PHASE SENSING BASED ON PLASMONIC METAMATERIAL FOR DETECTION OF OLIGONUCLEOTIDE SEQUENCES OF THE RPOB GENES OF MYCOBACTERIUM TUBERCULOSIS .

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ABSTRACT

Plasmonic metamaterials are capable to boost the refractive index sensitivity using phase down to single molecular detection limit. We designed optical components to demostrate phase sensing on tuberculosis DNA sequences detection model. Periodic array of 100-nm nanoparticles was prepared by e-beam lithography. Using the diffraction coupled resonance in reflection we registered phase jump using ellipsometer. We demonstrate that the phase sensitivity to the gold immobilization with P2 and further hybridization with T2 gene results overdrive the possibilities of standard SPR, taking into account the state of art phase detection methods. Comparing with SPR bio-detection methods, the use of nanostructure opens the possibilities of SERS-detection due to the strong nearfield enhancement in diffraction-coupled regime.

Keywords:

Metamaterial, biosensor, tuberculosis, phase

1.   INTRODUCTION

Surface plasmon resonance has became an industry standard technique for label-free biodetection over past decades[1]. Being incapable for detection of light-weight molecules in low concentrations (below 1 pg/mm^2, the use of phase curve was proposed as sensing parameter instead of commonly used intensity tracking[2]. This allowed to boost the detection limit to another 3 orders of magnitude. However the perfectly sharp phase jump with theoretically infinite sensitivity cannot be achieved on golden film due to instability of golden surface. The conditions of complete darkness accompanied with phase singularity can be met in periodic arrays of plasmonic nanoparticles[3]. The special design of those nanostructures allows one the get in the spectrum the point of complete darkness in asymmetric media conditions, where the resonance exists while the structure is in contact with different analytes. It was shown that the ultimate sensitivity using phase singularity can go down to 1 fg/mm^2.[4]

There was a considerable number of publications devoted to the understanding of coupling between localized plasmons in periodic arrays of nanoparticles[5,6]. It was shown that FOM of diffraction-coupled resonance can be maximized on case of phase registration at the point of complete darkness[7,8]. Not only

e-beam lithography arrays demonstrate excellent phase sensitivity but also the samples designed using Laser Induced Forward Transfer (LIFT) [9].

Although we used a standard-fabricated e-beam lithography sample, there was a special chemistry used for biosensing. First demonstrated on SPR [10] we use the same model for golden nanoparticle sample functionalization. We show that the phase shift during functionalization steps is several decades of degrees, so that with state-of-art phase sensing methods [11] we could detect much smaller fractions of oligonucleotides than with conventional amplitude SPR detection.

2.   RESULTS AND DISCUSSIONS

2.1 Sample fabrication

The sample was prepared using e-beam lithography on borosilicate glass. Particles were arranged on periodic arrays with 325 by 314 nm unit cell with overall size of 100x100 um. Individual nanoparticle had a semispherical shape with height and diameter of about 100 nm. Adhesion of gold with glass was enhanced by 3-nm chrome interface. Topographic images of resulting structure were done by NT-MDT AFM setup using 10-nm Si tip. Optical measurements were performed using Woollam M2000 various angle spectral ellipsometer with Xe lamp source focused into 30 um2 probe beam. The dry sample was illuminated from the face side while the in the fluid cell the sample was flipped so that probe beam entered from the bare glass side. The reflected light was collected by fiber spectrometer coupled with the phase sensor of ellipsometer.

Figure 1 represents the spectra of reflection for air and water environments. One can see the broad peak in p-polarization corresponding to localized plasmon scattering of individual nanoparticles with a narrow dip 600 nm due to diffractive plasmon coupling. FWHM of the dip is about 20 nm and its central wavelength corresponds to the Rayleigh cutoff frequency in the air for 325 nm nanoparticle period. The same result was obtained by FDTD simulation of periodical array of golden nanoparticles on glass (simulation curve on Fig.1a). Once we change the air environment to water, collective resonance moves to 550 nm where the intensity drops to zero (Fig1b) giving a nice phase jump on Fig1c. The nature of this resonance is not clear since the Rayleigh anomaly moves in the IR direction, neither it can be simulated. The only fact pointing to collective nature of this deep is the spectral sensitivity as we can see on Fig.2. As it was discussed in the previous papers, the amplitude and sharpness of the phase jump is not related to the resonance width but depends on the minimal reflection intensity. As we can see on Fig 2 phase jump bandwidth is only 10 nm while the intensity resonance width is 50 nm. In the ideal case the zero reflection is accompanied with infinitely sharp phase jump (dotted lines represent the momentary ellipsometric signal prior to temporal averaging). In our case due to temporal and spectral noise in the ellipsometer the phase curve is smoothed.
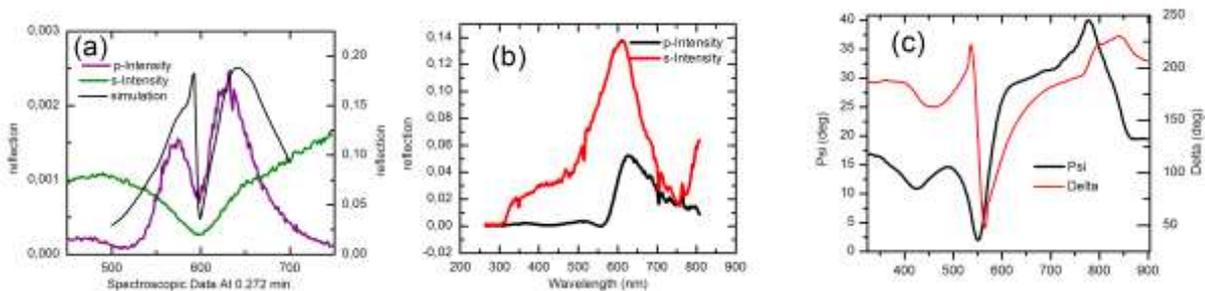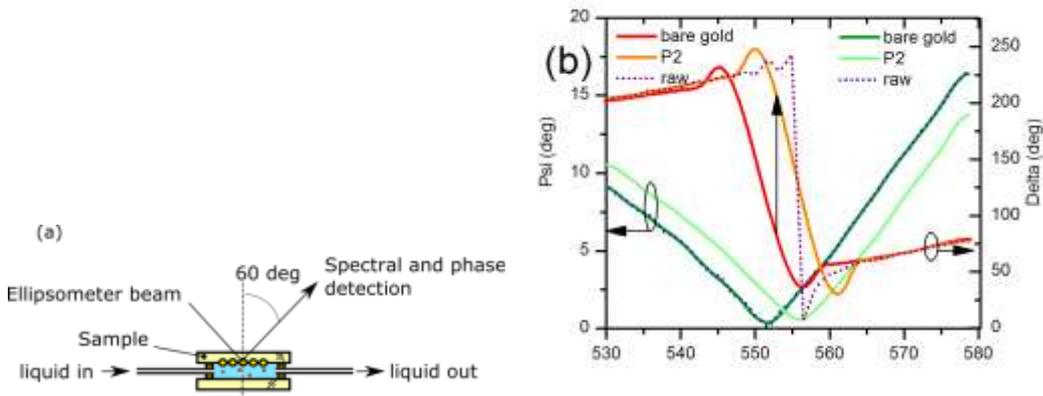

Figure 1.

Fig. 2. Scheme of experiment (a) and spectral and phase shift during P2 functionalization (b).

2.2 Sample functionalization

The experiment was done with the same protocol as in ref.[6] but without T2 and TN genes. So the fresh sample was immobilized with 1uM P2 solution in 0.5M KH2PO4 aqueous solution for 60 minutes. The solution was injected into the flow cell for 2 minutes and then stopped for 58 minutes. On the Figure 3a one can see the rise of phase at 554 nm wavelength from 75 to 200 degrees during immobilization period 40-80 min. After rinse by the same solution the signal falls to 150 degrees that means removal of non-immobilized oligonucleotide molecules. To block the non-functionalized golden sites we used 1 mM aqueous solution of 1-mercapto-6-hexanol (MCH). The signal has raised up to 220 degrees since, according publications , the molecules of P2 prop up from the surface of gold. This signal sustains after another rinse with the solution. The next dramatic change of phase occurs due to the change of the solvent from KH2PO4 to 2xSSC aqueous solution.

Figure 3 depicts the process of hybridization between P2 and 100 nM T2, the complementary target. The maximum phase jump obtained during 30 minutes experiment – 15 degrees. This is the only experiment we tried, but this is enough to estimate the detection limit using phase and more sensitive and less-noisy instruments. The same concentration of T2 in SPR experiments gave the shift of 9 pixels in [6].

While the physical detection limit for SPR setups is typically 1 pixel (10-6 RIU, 1nM T2 can be detected), the phase sensors based on elastic phase modulation are capable for phase detection of 5x10-3 deg. The use of this detector would allow to detect as small as small as 30 pM of T2.

The minimum concentration of DNA sequence detection reported in [12] is 200 pM which was obtained using custom built phase contrast setup. This result along with our one demonstrates the feasibility of plasmonic nanostructures and phase detection in biosensing experiments. Even using quite noisy in time ellipsometer setup, we could demonstrate a decent sensitivity, but phase contrast methods with no doubt
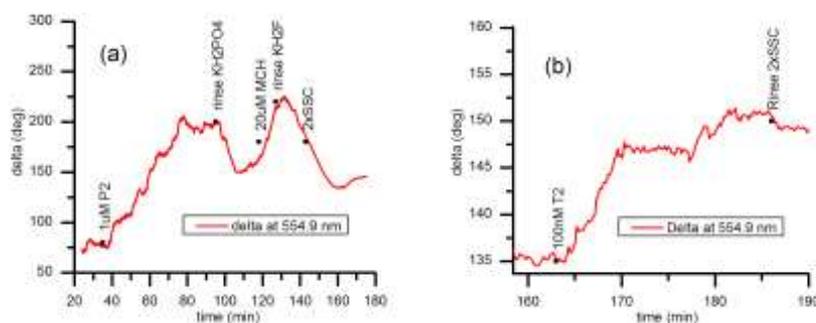

Fig. 3. Phase sensing curves for T2 immobilization (a) and P2 hybridization (b).

3.   CONCLUSION

We have demonstrated the capabilities of phase sensing using metamaterial array of nanoparticles in tuberculosis oligonucleotides detection experiment. Nanoparticle array used in the experiments demonstrates an excellent sensitivity to tuberculosis oligonucleotides hybridization. With state of the art phase detection methods this structure can feature as low as 30 pM concentrations of complementary genes sequences. Featuring the possibility of surface enhanced Raman spectroscopy as the second channel of detection, this kind of sensors can demonstrate even more prominent performance.

ACKNOWLEDGEMENTS

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